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Mutagenicity and Cytotoxicity of Coal Fly Ash from Fluidized-Bed and Conventional Combustion

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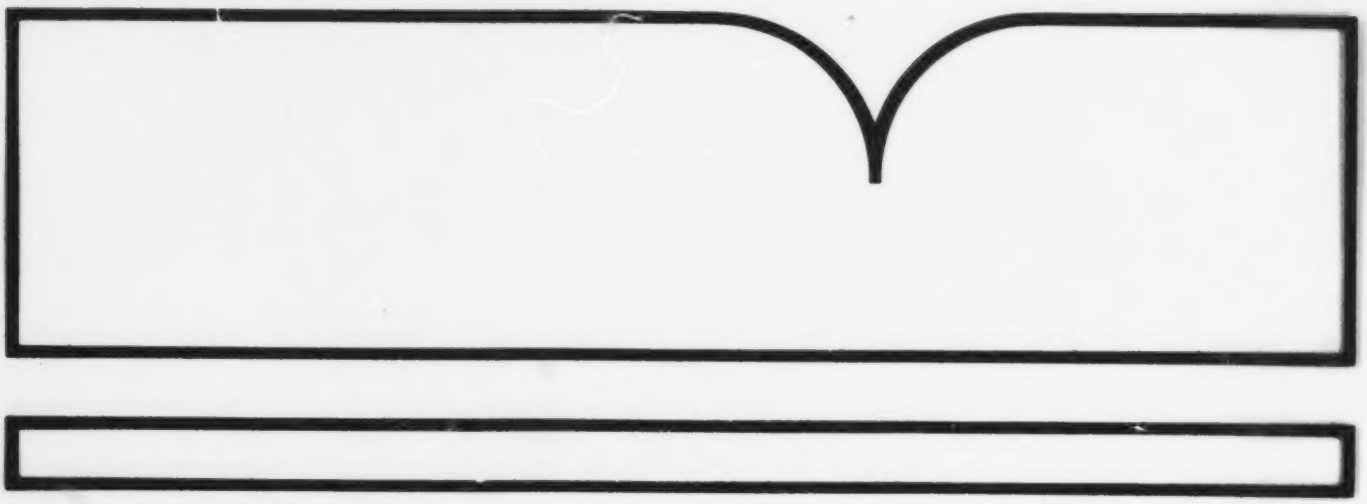

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16. ABSTRACT

Two respirable coal fly-ash samples (3 um) were evaluated for physical properties chemical composition, and biological activity, including cytotoxicity and mutagenicity. These two fly-ash samples were collected from a pressurized fluidized-bed combustion miniplant and from a conventional-combustion power plant, both burning eastern coal. All samples collected by a fabric filter downstream of the plant particulate control devices at 165 C (for fluidized-bed combustion fly ash) or 130 C (for conventional combustion fly ash). The two coal fly-ash samples were found to have different properties. Scanning electron microscopy illustrated a high degree of irregularity in fluidized-bed combustion fly ash and spericity in conventional-combustion fly ash. Elemental analysis showed that fluidized-bed combustion fly ash was higher in calcium, iron, chromium, nickel, selenium, and zinc, and conventional combustion fly ash was higher in aluminum, silica, arsenic, vanadium, and lead. Both fly ash samples were toxic in two mammalian cell systems, rabbit alveolar macrophages and Chinese hamster ovary cells in vitro, with fluidized-bed combustion fly ash showing higher toxicity in both assay systems. The rabbit alveolar macrophage system was a more sensitive assay than the Chinese hamster ovary system. For measuring the cytotoxicity of fly ash, the most sensitive parameters were adenosine triphosphate in the rabbit alveolar macrophage system.

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MUTAGENICITY AND CYTOTOXICITY OF COAL FLY ASH FROM FLUIDIZED-BED AND CONVENTIONAL COMBUSTION

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Two respirable coal fly-ash samples (≤3 μm) were evaluated for physical properties, chemical composition, and biological activity, including cytotoxicity and mutagenicity. These two fly-ash samples were collected from a pressurized fluidized-bed combustion miniplant and from a conventional-combustion power plant, both burning eastern coal. All samples were collected by a fabric filter downstream of the plant particulate control devices at 165°C (for fluidized-bed combustion fly ash) or 130°C (for conventional combustion fly ash). The two coal fly-ash samples were found to have different properties. Scanning electron microscopy illustrated a high degree of irregularity in fluidized-bed combustion fly ash and sphericity in conventional-combustion fly ash. Elemental analysis showed that fluidized-bed combustion fly ash was higher in calcium, iron, chromium, nickel, selenium, and zinc, and conventional combustion fly ash was higher in aluminum, silica, arsenic, vanadium, and lead. Both fly-ash samples were toxic in two mammalian cell systems, rabbit alveolar macrophages and Chinese hamster ovary cells in vitro, *with fluidized-bed combustion fly ash showing higher toxicity in both assay systems. The rabbit alveolar macrophage system was a more sensitive assay than the Chinese hamster ovary system. For measuring the cytotoxicity of fly ash, the most sensitive parameters were adenosine triphosphate in the rabbit alveolar macrophage system and viability index in the Chinese hamster ovary system. Intact fluidized-bed combustion fly-ash particles proved mutagenic only in tester strains TA98 and TA1538 without metabolic activation in the Ames* Salmonella typhimurium *plate incorporation assay. For conventional-combustion fly ash, no mutagenicity was detected in bioassay of the whole particles. A series of organic solvents with differing polarities was employed to identify the most efficient solvent for removing mutagens from coal fly ash. Soxhlet extraction yielded more extractable mass from fluidized-bed combustion fly ash than from conventional-combustion fly ash. The extracts of fluidized-bed combustion fly ash were found to be mutagenic when dichloromethane, acetone, or cyclohexane were employed. These samples contained direct-acting, frameshift mutagens. Much lower mutagenic activity was found in dichloromethane and acetone extracts of conventional-combustion fly ash.*

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INTRODUCTION

It has been estimated that coal has already supplied about 25% of the world's energy and can provide 50–66% of additional energy needs in the next 20 years (Wilson, 1980). The use of coal as a national energy source in this country is predicted to increase substantially, primarily through conventional and alternative coal technologies. Of the alternative technologies, fluidized-bed combustion (FBC) offers the potential of burning abundant high-sulfur or other low grade coal without the additional cost of flue gas desulfurization required in conventional-combustion (CC) plants to meet the emission control standards for sulfur dioxides (Robison, 1979). The mixture of coal and limestone in a fluidized bed can result in the adsorption of sulfur oxides by the limestone. The lower combustion temperature (800–1000°C) of FBC compared to CC (1400–1700°C) also reduces nitrogen oxide emissions (Henschel, 1978; Fennelly et al., 1977). With pressurized atmospheres (6–10 atm), FBC can maintain higher air density in the fluidized bed during combustion, thus requiring a smaller unit and reducing the capital and operating cost (Abelson and Lowenbach, 1977). It has been speculated, however, that FBC's low temperature combustion might produce higher levels of polycyclic organic matter (POM) in effluents and deposits on the surface of the emitted coal fly-ash particles (Kubitschek and Williams, 1980).

The mutagenicity of coal fly-ash extracts has been explored in recent years. Chrisp et al. (1978) and Fisher et al. (1979) reported that organic and serum extracts of western coal fly ash were mutagenic. Findings of mutagenicity in the organic extracts of coal fly ash have been shown in the work of Clark and Hobbs (1980) on an experimental 18-in FBC combustor and by Kutischek and Williams (1980) on a bench-scale 6-in model. One environmental health issue raised by FBC particulate emissions concerns their fineness and respirability compared to CC particles. The finer particles reside longer in the atmosphere, thus increasing their chances of being inhaled. When inhaled, they deposit efficiently in the alveolar region.

Alveolar phagocytic macrophages have been widely used to study the toxicity of inhaled contaminants both *in vitro* and *in vivo* (Allison, 1970; Waters et al., 1974, 1975a; Aranyi et al., 1979; Huisingh et al., 1977; Bingham et al., 1972; Coffin et al., 1968). These scavenger cells in the lung play an essential role in defending the host against inhaled toxicants, especially particles (Green, 1969). The viability and cellular functions of alveolar macrophages, however, can be adversely altered by exposure to environmental particles. Chinese hamster ovary (CHO) cells also are well characterized and able to phagocytize particles. This CHO cell system has been employed to evaluate the cytotoxicity and mutagenicity of toxicants (Hsi et al., 1975; Garrett et al., 1980). Both rabbit alveolar macrophage (RAM) and CHO cells were used in this study to evaluate the cytoxicity of the fly-ash particles.

The *Salmonella typhimurium* plate incorporation test developed by Ames et al. (1975) has been widely used to evaluate the potential mutagenicity and carcinogenicity of pure chemicals and complex environmental samples. This rapid and sensitive assay detects substances that cause frameshift and base-pair mutations in histidine-requiring mutants that revert to prototrophy as a result of exposure to the mutagen. The *Salmonella typhimurium* bioassay was used in this study to evaluate the mutagenicity of the fly-ash samples.

This study investigated the physical characteristics, chemical properties, mutagenicity, and cytotoxicity of respirable FBC and CC fly ash. Sample preparation and bioassay methods were evaluated for subsequent studies on fly-ash samples collected at multiple sites of the fluidized-bed combustion sources, including a commercial unit and other conventional combustion sources.

MATERIALS AND METHODS

Sample Collection

The fly ash from the combustion of Pennsylvanian eastern bituminous coal (2% sulfur, 8% ash) was collected from an experimental pressurized FBC miniplant at Exxon, Linden, N.J. (Kindya et al., 1980), sponsored by the U.S. Environmental Protection Agency. The internal diameter of the combustor was 33 cm (13 in), and the height of the vessel was 9.8 m (32 ft). The coal-firing capacity of the plant was 1.8 MW (6.3×10^6 Btu/h). Combustion temperature was about 870–900°C; pressure was 9 atm. Heat of combustion was removed by water-cooled tubes located in the fluidized bed. The sorbent for the FBC bed was dolomite with a Ca/S ratio of 1.25. The ash was collected, after three stages of high-efficiency conventional cyclones, by a trailer-mounted fabric filter treated with a slipstream of flue gas. The trailer was located outside the combustion plant. The FBC fly-ash sample was collected at ~165°C during a steady state of plant operation, representing 6 d of collection.

For the CC source sample, coal fly ash from the combustion of Alabaman eastern bituminous coal (1–2% sulfur, 11% ash) was collected downstream of an electrostatic precipitator from a commercial coal-fired power plant in the southern United States. This ash was collected by a fabric filter inside a high-volume source sampler at ~130°C (Cushing and Smith, 1979). The source sampler contained a cyclone to remove larger particles upstream from the fabric filter.

Sample Preparation

Organic solvents of differing polarity were evaluated to determine their efficacy in removing mutagens from the fly-ash particles. The solvents investigated were dichloromethane, acetone, cyclohexane, and methanol of spectrograde quality (Burdick and Jackson Laboratories, Inc., Muskegon,

Mich.). The fly-ash particles were Soxhlet-extracted for 24 h. Mutagenicity bioassays were performed following the removal of the solvent by nitrogen evaporation and addition of dimethyl sulfoxide.

Chemical Analysis

Carbon, hydrogen, and nitrogen contents of the particles and their organic extracts were analyzed by a PE-240 elemental analyzer (Jungers et al., 1981). Anion analysis for fluoride, chloride, nitrate, and sulfate was conducted by ion chromatography (Dionex) (Jungers et al., 1981). Trace elements were analyzed by spark-source spectrometry according to Daughtrey and Bostick (1977), and neutron activation according to Weaver (1978).

Scanning Electron Microscopy

Physical characteristics of both fly-ash samples were examined by scanning electron microscopy. Samples were shaken vigorously and small aliquots placed onto conductive copper tape on aluminum stubs. Stubs coated with platinum using a magnetically controlled sputter coater (Ingram et al., 1976) were examined at 20 kV in a scanning electron microscope (Shelburne et al., 1976).

Cytotoxicity Assay Using Rabbit Alveolar Macrophages and Chinese Hamster Ovary Cells

Alveolar macrophages were obtained from New Zealand white rabbits (Charles River Breeding Laboratories, Wilmington, Mass.) by tracheobronchial lavage (Myrvik et al., 1961). Rabbits weighing 1.5-2.0 kg were sacrificed by the injection of 150 mg of sodium pentobarbital into the marginal ear vein. The lungs were lavaged *in situ* with warm saline through a catheter incised into the trachea. The macrophage yield consisted of an average of 20 to 30 million cells per rabbit. Differential cell counts were performed using Wright-stained smears. Cellular composition of acceptable lavage fluid for these studies was about 97% macrophages, 2% lymphocytes, 0.5% polymorphonuclear leukocytes, and 0.5% eosinophils.

The cells were centrifuged at 350 g for 15 min at 4°C, then washed with normal saline. The cells were resuspended in Medium 199 with Hanks salt. The medium was supplemented with 20% heat-inactivated fetal calf serum, 100 units/ml penicillin, 100 μg/ml kanamycin, and 100 μg/ml streptomycin (GIBCO, Grand Island, N.Y.). Cell number and viability were determined by a hemocytometer using trypan blue dye exclusion technique (Weissbecker et al., 1969). Only cells with viability higher than 90% were used. Cell density was adjusted to 2.0×10^6 cells/ml medium.

The procedure used for cytotoxicity assay was that of Garrett et al. (1981a). The parameters employed to measure toxicity were viability, viability index, adenosine triphosphate (ATP), and protein synthesis. The coal fly-ash particles were suspended in Medium 199 containing no serum

and preincubated with the medium for 20 h at 37°C on a Bellco rocker platform (12 oscillations/min). The RAM cell suspension (1 ml of 2×10^6 cells/ml) containing 20% FCS was added to 1 ml particulate suspension in Costar six-well cluster dishes. One duplicate set of wells was prepared for protein synthesis determination. One microcurie of tritiated leucine was added to each of these wells. The cultures were incubated on a Bellco rocker platform (12 oscillations/min) for 20 h at 37°C in a humidified atmosphere of 5% CO_2 and 95% air.

At the end of the incubation period, the unattached cells were removed and retained. The attached cells were trypsinized and combined with the unattached cells. A combined cell suspension of 0.1 ml was used for ATP determination, and 0.5 ml cell suspension was used for viability and cell number determinations. Viability was expressed as percentage of viable cells counting a minimum of 400 cells with a hemocytometer. The viability index is a parameter that defines the combined effects of a test compound on viability and cell number as:

$$\text{Viability index} = \text{viability (\%)} \times \frac{\text{number of cells in test culture}}{\text{number of cells in control culture}}$$

The ATP was determined with a Dupont Model 760 Luminescence Biometer using the luciferin-luciferase reaction (Waters et al., 1975b), calculated as femtograms (fg) per milliliter and expressed as a percent of control.

Procedures for protein synthesis determination were modified from the method of Gautschi et al. (1973). Deoxycholate (5%) in 0.5 ml solution was added to lyse the cells; 0.1 ml of the cell lysate was placed on glass-fiber filters for precipitation of the protein by adding 5 ml of a 4°C 10% trichloroacetic acid solution containing 10 mM leucine. After 30 min the filters were washed three times with 5% trichloroacetic acid and then with 95% ethanol. The air-dried filters were transferred to counting vials. Aquasol (4 ml) was added, and the protein on the filters was counted by a liquid scintillation spectrometer. Protein synthesis was expressed as percent of the control culture.

The CHO cells were also employed for cytotoxicity assay and were obtained from the American Type Culture Collection. The CHO cells were maintained in Ham's F-12 medium with 10% FCS. The cells were cultured at 37°C in the flasks and subcultured using 0.25% trypsin twice weekly. The cytotoxicity assay using CHO cells was carried out with the same procedure as for the RAM system, except that only 0.5 million CHO cells/ml were seeded initially in each well to allow cell division during incubation.

The toxicity assay was performed in a dose-response fashion using 7 concentrations ranging from 10 to 1000 μg/ml. Three replicates for each dose of the test sample and five replicates for control culture were

conducted in each experiment. At least two experiments were performed for each sample and the data were pooled for computation. The dose-response data were also used to estimate the EC50 values, which were defined as sample concentrations required to reduce cell viability or other cell functions to 50% of the control values. Data were mathematically transformed and then fitted to a straight line by the method of least squares. Sample concentrations (μg/ml) were in natural logarithms, and cell viability was transformed to the arc sine of the square root of the fraction of viable cells. The ATP data in percentage of control were fitted to a straight line with the logarithm of the doses. The EC50 values were then obtained from the simple regression line after these transformations. The details of the data analysis were reported by Garrett and Stack (1980).

Ames Microbial Mutagenicity Bioassay

Both the particles and the organic extracts of the fly-ash samples were bioassayed for mutagenicity using the *Salmonella typhimurium* plate incorporation method (Ames et al., 1975) with the following modifications. The standard minimal histidine was added to the plating media, instead of the soft agar overlay. The plates were incubated for 72 h (Claxton et al., 1979). *Salmonella typhimurium* tester strains were obtained from Dr. Bruce Ames. For metabolic activation, a 9000-g supernatant (S9) of a liver homogenate was prepared using Arochlor-1254-induced male rats (Charles River CD-1) (Ames et al., 1975). The studies were performed at least in duplicate at five doses or more, depending on the amount of sample available. The fly-ash particles were directly suspended in the top agar at a concentration range of 1 to 25 mg. All five tester strains of TA98, TA100, TA1535, TA1537, and TA1538 were employed with and without metabolic activation. The organic extracts prepared from the particles (see sample preparation section) were dissolved in dimethyl sulfoxide at a concentration of 2 mg/ml and bioassayed at a concentration range of 20 to 600 μg/ml. With a limited amount of these samples available, the organic extracts were tested only with TA98 with and without metabolic activation. Tester strain TA98 has been found in this study and by Kubitschek and Williams (1980) to be the most sensitive strain for detecting the mutagenicity of coal fly ash.

Negative and positive controls were conducted with each experiment. For the experiments with metabolic activation, 2-anthramine was used as the positive control for each strain. For the experiments without metabolic activation, the positive controls were 2-nitrofluorene for TA98 and TA1538, 9-aminoacridine for TA1537, and sodium azide for TA100 and TA1535. A sample was considered positive if it showed a maximum response 2 times greater than the spontaneous rate and if it gave a positive linear dose-response. The linear portion of the dose-response curve was used to calculate a linear regression line. The slope of the linear regression was reported as revertants per weight unit of samples tested.

RESULTS

Physical Characteristics

Electronmicrographs in Figs. 1 and 2 illustrate the physical characteristics of the FBC fly ash and CC fly ash. In both samples, the particles formed small aggregates. The size distribution by electron microscopy analysis (Table 1) shows both samples to be primarily less than 3 μm. FBC fly ash contained a higher percentage of fine particles, with 99.1% being below 1.7 μm. The geometric mean diameter for FBC fly ash was 0.54 μm, and 1.05 μm for CC fly ash.

The CC fly ash in this study was glassy, spherical, with fiber-like material on the surface, while FBC fly-ash particles were flaky, irregular, and rocky.

Chemical Analysis

Results of elemental analysis using spark source mass spectrometry and neutron activation are presented in Table 2. The primary elements in both FBC and CC fly ash were aluminum, silica, iron, and calcium. The two

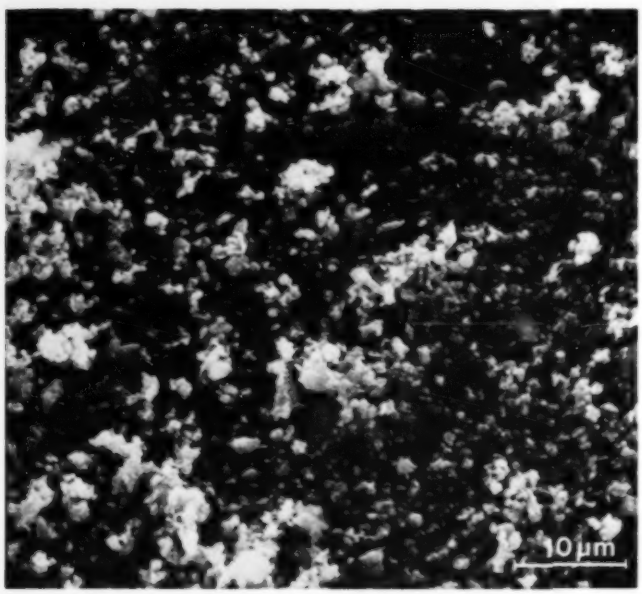

FIGURE 1. Scanning electron micrograph of coal fly ash from a pressurized FBC miniplant, ×1737.

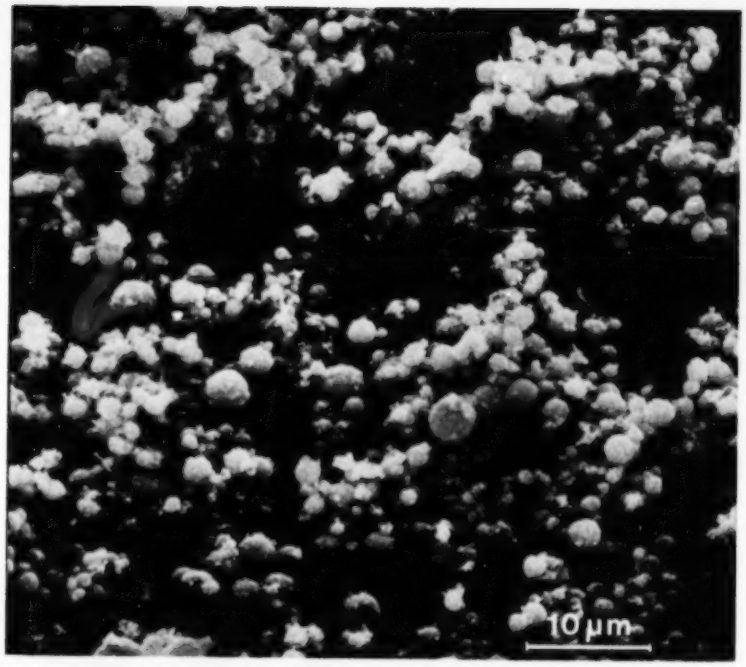

FIGURE 2. Scanning electron micrograph of coal fly ash from a CC power plant, ×1737.

TABLE 1. Particle-Size Analysis of Fly Ash by Scanning Electron Microscopy

	Particle diameter (μm)						
Analysis	0–0.42	0.42–0.83	0.83–1.7	1.7–2.5	2.5–3.3	3.3–4.2	4.2–5.0
			CC fly ash				
Count[a]	17	111	164	59	15	3	2
Percent[b]	4.6	29.9	44.2	15.9	4.0	0.8	0.5
Cumulative %[c]	4.6	34.5	78.7	94.6	98.6	99.4	100
			FBC fly ash				
Count[a]	106	169	68	3			
Percent[b]	30.6	48.8	19.7	0.9			
Cumulative %[c]	30.6	79.4	99.1	100			

[a] Actual particle count.
[b] % Particles counted found in this size range.
[c] % Particles in and less than this size range.

TABLE 2. Elemental Analysis of Fly Ash By Spark Source Mass Spectrometry[a] and Neutron Activation Analysis[b]

	FBC fly ash (μg/g)		CC fly ash (μg/g)	
Element	SSMS	NAA	SSMS	NAA
Al	–	58,027	–	136,612
Si	60,000	–	137,000	–
Fe	–	130,008	–	88,168
Ca	48,600	–	22,300	–
V	108	210	320	835
Cr	–	3,221	–	1,375
Mn	650	495	250	488
Co	26	103	25	93
Ni	960	–	48	–
As	49	90	2,140	3,653
Se	340	494	16	137
Cd	17	<20	19	<20
Zn	–	3,895	–	1,957
Pb	125	–	273	–
Th	33	20	26	37
U	55	6	68	22

[a]Precision ± 30%.
[b]Precision ± 2%.

fly-ash samples differed in their concentrations of trace elements. FBC fly ash contained higher concentrations of chromium, nickel, selenium, and zinc. CC fly ash contained higher concentrations of arsenic, vanadium, and lead.

Table 3 presents carbon (C), hydrogen (H), and anion analysis of fly ash comparing direct analysis of fly ash with acetone, methanol, cyclohexane, and dichloromethane extracts. These data show that FBC fly ash and the solvent extracts contained much higher concentrations of chloride ions. The FBC sample also showed somewhat higher concentrations of fluoride ions. The carbon content of two fly-ash samples was similar. The FBC fly ash contained more organic-solvent-extractable mass than the CC fly ash. All the organic solvents removed 2 to 3 times more mass from FBC particles than from CC particles. The most polar solvent, methanol, removed substantially more inorganic sulfates than other solvents. With the exception of methanol, acetone removed the most extractable matter from both fly ash samples, followed by dichloromethane and cyclohexane.

Cytotoxicity

Particles of both FBC fly ash and CC fly ash were toxic in RAM and CHO systems *in vitro* after 20 h of cell exposure. Figures 3 and 4 show effects of both fly-ash samples in RAM and CHO cells on viability,

TABLE 3. Elemental and Anion Analysis and Extractable Mass of Fly Ash

Sample[a]	Extractable mass (%)	C (%)	H (%)	F^- (μg/g)	Cl^- (μg/g)	SO_4^{-2} (μg/g)
FBC fly ash	–	1.2	0.7	408.6	1,032.8	11,816.0
Dichloromethane ext.	0.06	–	–	–	–	–
Acetone ext.	0.30	26.3	5.3	1.9	485.0	61.7
Methanol ext.	7.0	5.1	3.7	178.3	672.7	3,110.4
Cyclohexane ext.	0.04	–	–	–	–	–
CC fly ash	–	1.0	0.2	232.4	53.8	13,449.0
Dichloromethane ext.	0.02	–	–	–	–	–
Acetone ext.	0.11	38.3	5.9	0.8	22.6	12.7
Methanol ext.	2.55	3.2	4.4	120.5	38.9	5,777.7
Cyclohexane ext.	0.01	–	–	–	–	–

[a]Dichloromethane and cyclohexane extracts were not available for the elemental and anion analysis because of their low yield.

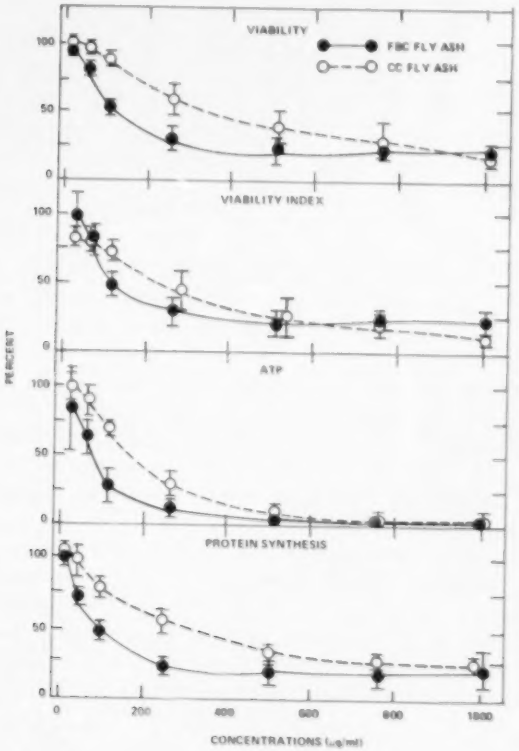

FIGURE 3. Toxic effects of fly ash on RAM cells after a 20-h exposure *in vitro*. Data presented are mean ± standard deviation of three experiments (nine replicates). Viability is calculated in percent viable cells. ATP (in fg/ml) and protein synthesis (in dpm) are expressed in percent control.

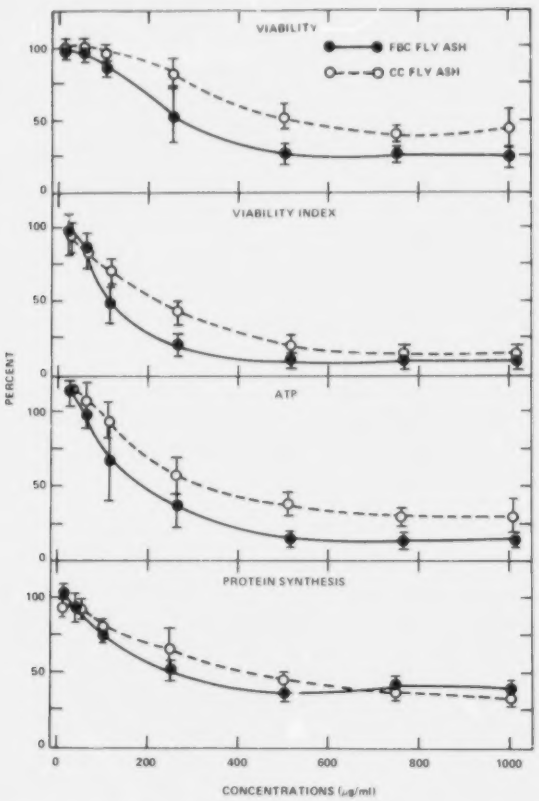

FIGURE 4. Toxic effects of fly ash on CHO cells after a 20-h exposure *in vitro*. Data presented are mean ± standard deviation of two experiments (six replicates). Viability is calculated in percent viable cells. ATP (in fg/ml) and protein synthesis (in dpm) are expressed in percent control.

viability index, ATP, and protein synthesis. All the toxicity parameters in RAM and CHO cells for both types of fly-ash particles started to show significant decreases ($p < 0.05$) at concentrations of 250 μg/ml or below, as compared to the control.

Table 4 summarizes the effective concentrations (EC50) required to reduce cell numbers or cell functions to 50% of the controls for the two samples in the RAM and CHO systems. The more toxic samples had lower EC50 values. FBC fly ash generally had lower EC50 values than CC fly ash. The relative sensitivity of RAM and CHO assay was compared in these two samples. In the RAM assay, both samples had lower EC50 values than the CHO assay for all the parameters except viability index. Both fly-ash samples decreased cellular ATP content to 50% of the control values at

TABLE 4. Cytotoxicity of Fly Ash

Cell system	Sample	EC50 (μg/ml)[a] % Viability	Viability index	ATP	Protein synthesis
RAM	FBC	140 (120-162)	127 (104-152)	56 (43-70)	127 (109-147)
	CC	328 (274-399)	217 (176-270)	152 (135-172)	368 (309-445)
CHO	FBC	297 (243-370)	115 (94-139)	176 (142-217)	298 (252-357)
	CC	781 (578-1133)	158 (133-187)	290 (237-362)	485 (365-687)

[a]EC50 values were obtained from simple regression. The numbers in parentheses are 95% confidence intervals.

lower concentrations than any other parameters measured in the RAM system. In the CHO assay, however, the viability index was decreased to 50% of the control values at lower concentrations of fly ash than the other parameters examined.

Mutagenicity

Figure 5 shows the comparative mutagenicity of FBC and CC fly-ash particles in the Ames *Salmonella typhimurium* assay in tester strain TA98. FBC fly-ash particles showed a positive mutagenic response in TA98 without S9 metabolic activation. The FBC fly-ash particles also showed mutagenic activity in TA1538 without S9. The specific activity was 3.32 revertants/mg FBC fly ash in TA98 and 3.31 revertants/mg FBC fly ash for TA1538 without S9. Although the mutagenic activity of FBC fly ash did not reach more than 2 times the spontaneous rates in TA100, it did show a tendency to increase with the dose. When FBC fly-ash particles were tested directly in the agar overlay using TA98, a clearing of the background bacterial lawn appeared around aggregated particles (see Fig. 6). This clearing or toxicity was apparently due to leachable material from the aggregated particles. Revertant colonies tended to appear near these rings, as shown in the Fig. 6. Metabolic activation decreased the mutagenic activity of the FBC fly ash in TA98 and TA1538. FBC fly ash showed no significant mutagenic response in TA1537 and TA1535 with and without metabolic activation. These results indicated that FBC fly ash contains direct-acting, frameshift mutagens. The CC fly-ash particles showed no mutagenicity in all five tester strains.

The mutagenicity dose-response of the organic extracts of FBC and CC fly ash in TA98 with and without activation is shown in Figs. 7 and 8. The slope obtained from the linear regression analysis of the dose response data is shown in Table 5. Metabolic activation decreased the mutagenicity of both samples. Organic extracts of FBC fly ash consistently displayed higher mutagenicity than CC fly ash. Among the solvents examined, dichloromethane extraction resulted in the highest mutagenic activity

(3.07–9.43 revertants/μg, without activation). Less mutagenic activity was observed in the solvent-extractable material removed with acetone (1.34–4.45 revertants/μg, without activation) and cyclohexane (0.10–1.71 revertants/μg, without activation). Methanol extraction of both samples yielded nonmutagenic material.

DISCUSSION

In this study, the respirable fly ash from a pressurized FBC miniplant and from a conventional utility boiler, both burning eastern coal, were investigated. The fly-ash samples were collected by a fabric filter at 165°C and 130°C, respectively.

The objective in this study was to evaluate the physical and chemical properties of these particles, as well as their biological activity. Since these

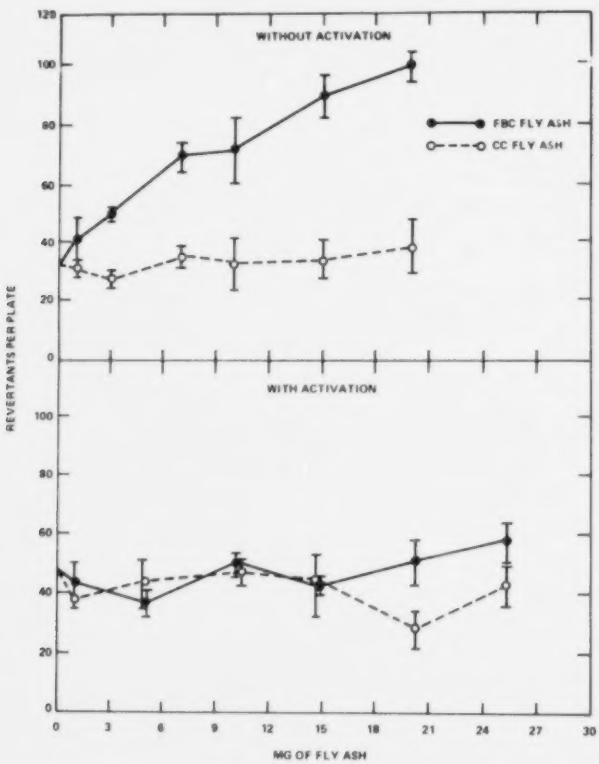

FIGURE 5. Mutagenicity of fly ash particles in *Salmonella typhimurium* TA98. Data presented are mean ± standard deviation of three replicates.

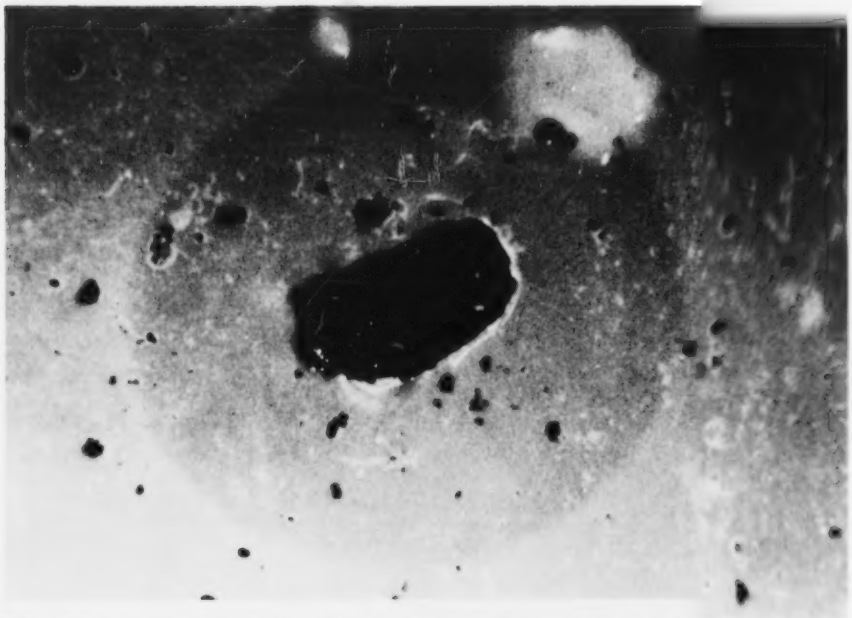

FIGURE 6. Leachate from fly ash aggregates in agar medium. The white colonies lo[illegible]cated on the edges of the ring are revertants of *Salmonella typhimurium* TA98.

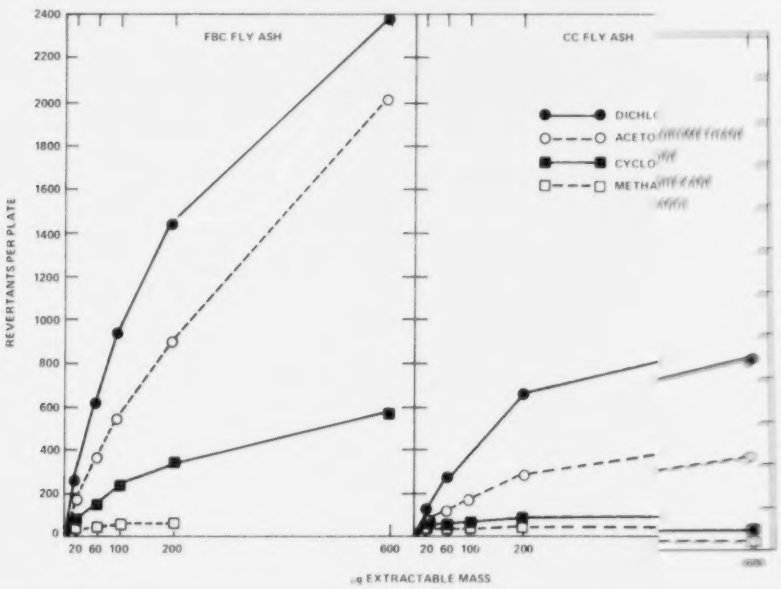

FIGURE 7. Mutagenicity of organic extracts of fly ash in *Salmonella typhimuriu[illegible]* TA98 without metabolic activation. Each point is an average of duplicates.

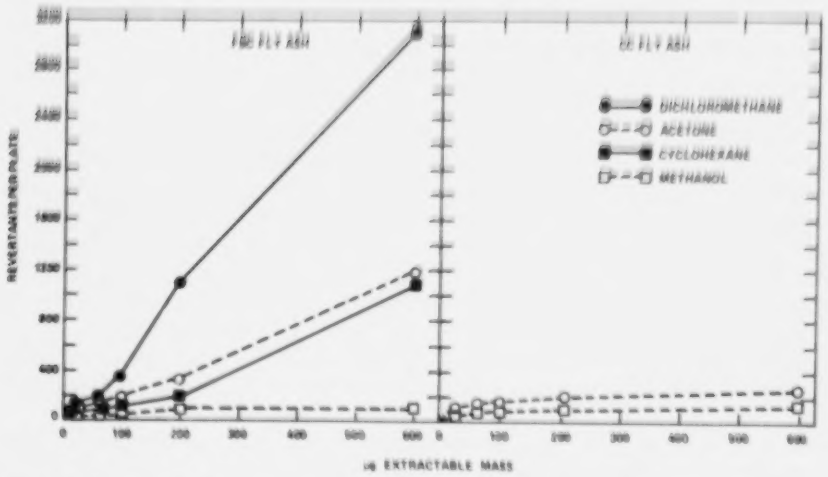

FIGURE 8. Mutagenicity of organic extracts of fly ash in *Salmonella typhimurium* TA98 with metabolic activation. Each point is an average of duplicates. Dichloromethane and cyclohexane extracts of CC fly ash were not available for testing as the yield of the extracts was too low.

TABLE 9. Mutagenicity of Fly Ash in the *Salmonella typhimurium* Plate Incorporation Assay Using TA98

Sample	Extraction solvent	% Extractable mass	Revertants/μg extractable mass[a]		Revertants/mg equivalent fly ash[b]	
			With activation	Without activation	With activation	Without activation
FBC fly ash	Dichloromethane	0.66	5.21	9.43	3.43	5.66
	Acetone	6.30	1.98	6.45	3.09	13.35
	Methanol	7.0	Neg[d]	Neg.	Neg.	Neg.
	Cyclohexane	0.64	0.87	1.71	0.35	0.68
	None	—	—	—	Neg.	3.92[c]
CC fly ash	Dichloromethane	0.02	—	3.07	—	0.61
	Acetone	0.31	0.93	1.36	0.47	1.47
	Methanol	2.55	Neg.	Neg.	Neg.	Neg.
	Cyclohexane	0.01	—	0.30	—	0.01
	None	—	—	—	Neg.	Neg.

[a]Revertants/μg organics obtained by computing the slope of the linear regression in the linear portion of the curve.

[b]Revertants/mg equivalent calculated by multiplying [revertants/μg organics] by [the number of μg organics/mg of fly ash].

[c]Revertants/mg of fly ash obtained by computing the slope of the linear regression from testing the particles directly.

[d]Neg, negative.

results may be dependent on sample preparation and bioassay methods, efforts were undertaken to explore alternative approaches.

Physical and Chemical Properties

Fly-ash particles provide a matrix for interactions of a great variety of substances during the combustion and emission process. Combustion temperature is an important factor that may determine the physical properties of fly ash. During the high temperature (1400–1700°C) combustion in a conventional coal-fired power plant, the major aluminosilicate in coal melts and condenses to form small, mostly spherical and glassy particles (Natusch, 1976). The FBC sample collected from a source with lower combustion temperature contained more irregular particles.

Both FBC and CC fly-ash samples are by-products of the combustion of low-sulfur eastern bituminous coal. The two eastern coal samples used were from different sources and may vary in some elemental composition. The two fly-ash samples contained the same four predominant major elements: aluminum, silica, iron, and calcium. The difference in the concentrations of some trace elements—e.g., arsenic and chromium—in these two fly-ash samples may have been due to the difference in coal. Whether the different combustion processes may contribute to the differences in the trace elements is not clear.

The surface characteristics of fly-ash particles are of great significance in environmental health. The nature of the particulate surface may determine the kind and degree of adsorption of the gaseous POM during cooling of the effluents in the stack and plume (Natusch, 1978; Rothenberg, 1980). When the particles are inhaled and deposited in the lung, the adsorbed materials may be slowly released, posing a potential health hazard. The porosity of FBC fly ash reported by Hanson et al. (1979) may contribute to the adsorption and release of organic substances from the fly-ash surface. The FBC fly-ash sample investigated here did contain more extractable organics than the CC fly ash, despite the fact that it was collected at somewhat higher (35°C) temperatures. Leachable material was also observed around the FBC fly-ash samples in agar medium, but was not seen in the CC fly ash. The fact that the FBC fly ash was mutagenic when tested in whole particles is further substantiation for the bioavailability of mutagens on the surface of fly ash.

Cytotoxicity

Fine particles of fly ash, having more specific area than large particles, can adsorb or condense various toxicants during the cooling process after combustion. These fine particles, especially the submicron size, can easily escape conventional control devices (Vandegrift et al., 1973) and be deposited efficiently in the lung (Fisher and Natusch, 1979).

Small coal fly-ash particles emitted from a power plant were reported by Davison et al. (1974) to have higher concentrations of trace elements—

e.g., As, Se, Cr, Zn, Pb, Cd, Ni—than the fly ash retained in the power plant. The trace elements were preferentially concentrated in the smaller particles in both the airborne particles and those retained in the plant. In this study, CC and FBC fly-ash samples containing fine emission particles were found toxic in the two mammalian cell systems tested. Further evidence correlating particle size and toxicity was recently provided by research using electron spectroscopy for chemical analysis (ESCA), which found arsenic to be predominant on the surface of CC fly-ash particles investigated here (Ranade and Shendrikar, 1981). The enrichment of arsenic on the surface of CC fly-ash particles appears to be associated with the cytotoxicity of this fly-ash sample.

EC50 values indicated that FBC fly ash was more toxic than CC fly ash. The differences in the content of trace elements and organic substances between the samples may account for the differences in cytotoxicity. The FBC fly ash had higher concentrations of extractable organics and certain trace elements (Cr, Ni, Se). The smaller size and more angular structure of the FBC particles may also have contributed to the higher toxicity observed.

Mutagenicity

Direct bioassay of intact FBC particles in the Ames test revealed the presence of direct-acting, frameshift mutagens. The CC particles, however, were not mutagenic upon direct bioassay. The use of a series of organic solvents to extract test material from these particles demonstrated the existence of mutagens in both FBC and CC fly ash.

The solvents that were most effective in removing organic rather than inorganic matter were also the most effective in removing mutagens. Since the mutagenic activity was observed in tester strains that respond to polycyclic frameshift mutagens, it is likely that the mutagens associated with these fly-ash samples were polycyclic organic compounds. Polycyclic organic matter is known to be emitted from coal combustion. In our recent studies, 1-nitropyrene, a nitropolycyclic aromatic hydrocarbon, was detected (130 ppb) in a similar FBC fly-ash sample collected from the same micriplant. The CC fly ash examined here also contained 1-nitropyrene (0.11 ppb) (Mumford and Lewtas, in press). The POM emissions from coal combustion increase as combustion efficiency decreases (Natusch, 1978). Studies suggest that decreasing the efficiency (measured by carbon monoxide) on an FBC combustor increases the mutagenicity of the fly ash emitted (Kubitschek et al., 1980).

As stated previously, the FBC sample contained more organic extractable matter than the CC fly ash, even though it was collected at a higher temperature. The organics associated with the FBC particles were also more mutagenic than the organics from the CC fly ash sample. The higher content of the organic matter and the associated mutagenicity in FBC fly ash may have resulted from less complete combustion in the fluidized-bed

combustor. FBC combustion temperatures are generally significantly lower (400–900°C) than conventional combustion temperatures.

The FBC sample was collected from a small pilot-scale fluidized-bed combustor. The mutagenicity or toxicity of the fly ash from this plant may be different from a large commercial-scale FBC unit. Development or modifications of FBC technology—e.g., increased recirculation of fly-ash catch, optimal staging of combustion air—may increase the combustion efficiency and decrease the POM emissions. The biological activity of fly ash from these improved units may differ from that reported here for an experimental FBC unit.

Sample Preparation and Bioassay Methods

In considering different methods of sample preparation of fly ash for bioassay, this study has shown that it is possible to detect the mutagenicity of fly ash by direct testing of particles without prior extraction in the Ames assay. This method provides a direct measure of the bioavailability of the mutagens under study and requires a much smaller sample. Direct bioassay of particles, however, produces findings of lower mutagenic activity and greater variation among the replicates than bioassay of the soluble organics that are removed from particles. The explanation for the decreased mutagenicity and greater variation may be the uneven distribution of the mutagens in the agar plate, as some mutagens adhere to the surfaces of the fly-ash particles.

In conjunction with direct testing of fly-ash particles, organic extraction by solvents can remove and concentrate the appropriate mutagens from the particles into concentrations that are readily detectable by the assay under study. The extracted mutagens can also be further fractionated to identify the biologically active components.

Since a spectrum of organic solvents may preferentially extract different constituents from particles, mutagens with varying composition and activity may be removed. Dichloromethane extraction yielded less mass than acetone or methanol from both coal fly-ash particles; however, this extract showed the highest mutagenic activity per microgram of the extractable mass. Acetone yielded more extractable mass and gave the highest total recovery of mutagenic activity when calculated per milligram equivalent of fly ash. Cyclohexane, the least polar solvent, resulted in the lowest extractable mass and in less mutagenic activity than dichloromethane or acetone. The lack of mutagenicity in the methanol-extracted matter may be attributed to the presence of high concentrations of inorganic substances. These findings are consistent with similar studies conducted with air particles. Jungers et al. (1981) found that among the seven solvent systems investigated, dichloromethane extraction appeared to preferentially remove mutagenic organics from the air particles along with minimal removal of inorganic matter.

Cytotoxicity test systems, including the RAM and CHO assay, have been employed to screen the toxicity of various industrial effluents (Duke et al., 1977). The RAM assay can be used to measure cytotoxic effects of airborne particles in lung cells (alveolar macrophages) that are the direct target of inhaled particles. Since the RAM assay employs primary cells obtained from animals, it is much more costly than cytotoxicity assays conducted with continuous cell lines, like the CHO assay. Previous studies have suggested that RAM and CHO cells respond in a similar manner to toxic particles (Garrett et al., 1981b). The cytotoxicity of the FBC and CC fly-ash samples was examined in both the RAM and CHO assays to further evaluate the comparative utility of these two assays.

Comparison of the EC50 values for both fly-ash samples in RAM and CHO cells showed that the RAM cells were generally more sensitive than the CHO cells to the toxicity of fly ash. The greater sensitivity of the RAM system may result from the highly migratory and phagocytic nature of the alveolar macrophages. Previous studies (Garrett et al., 1981a) have shown that phagocytic activity of latex spheres in RAM cells was greater than in CHO cells during the first 8-h period of cell culture. After 8 h, the phagocytic activity in these two cell systems was the same. ATP was the most sensitive index in the RAM assay. Only 56 μg FBC fly ash/ml and 152 μg CC fly ash/ml, were sufficient to reduce the ATP values to 50% of the control values. The fact that 140 μg FBC fly ash/ml and 328 μg CC fly ash/ml were needed to reduce percentage viability to 50% of the control values indicates that the cells' exposure to the particles affected cellular energy function well before cell death.

Since viability index reflects the combined effects on the total cell number and viability, fly-ash particles apparently affected CHO cell division at concentrations that did not affect cellular ATP and viability. The total cell number was in turn reduced, with the resultant low values of viability index at the end of the cell exposure. Use of the viability index in CHO cells, therefore, provides a toxicity measurement for these fly-ash samples, equivalent in sensitivity to measurement of ATP in the RAM cell assay.

SUMMARY

In summary, fly-ash samples from a pressurized fluidized-bed combustion miniplant were found to consist of submicron, irregular particles that were cytotoxic and contained bioavailable mutagens. The fly-ash emission sample from a conventional coal-fired power plant was found to consist of spherical particles that were also cytotoxic but less mutagenic.

The FBC fly ash investigated here was collected from an experimental miniplant and should not be considered representative of fly ash that may be obtained in the future from larger commercial-scale FBC plants. Further health and environmental assessment studies of coal fly-ash

samples collected at multiple sites, including commercial-scale fluidized-bed and other conventional combustors, are needed to evaluate the potential health effects of coal fly ash from both types of combustion technology.

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